Is there a way to curb benzodiazepine addiction?

Arnaud L. Lalive, Uwe Rudolph, Christian Lüscher, Kelly R. Tan

Department of Basic Neurosciences, Medical Faculty, University of Geneva, Switzerland
Laboratory of Genetic Neuropharmacology, McLean Hospital and Department of Psychiatry, Harvard Medical School, Belmont, MA, USA
Department of Basic Neurosciences, Medical Faculty, University of Geneva, Switzerland

Summary

Benzodiazepines are widely prescribed drugs used to treat anxiety and insomnia, induce muscle relaxation, control epileptic seizures, promote anaesthesia or produce amnesia. Benzodiazepines are also abused for recreational purposes and the number of benzodiazepine abusers is unfortunately increasing. Within weeks of chronic use, tolerance to the pharmacological effects can develop and withdrawal becomes apparent once the drug is no longer available, which are both conditions indicative of benzodiazepine dependence. Diagnosis of addiction (i.e. compulsive use despite negative consequences) may follow in vulnerable individuals. Here, we review the historical and current use of benzodiazepines from their original synthesis, discovery and commercialisation to the recent identification of the molecular mechanism by which benzodiazepines induce addiction. These results have identified the mechanisms underlying the activation of midbrain dopamine neurons by benzodiazepines, and how these drugs can hijack the mesocorticolimbic reward system. Such knowledge calls for future developments of new receptor subtype specific benzodiazepines with a reduced addiction liability.

Key words: benzodiazepines; addiction; ventral tegmental area; GABA\textsubscript{A} receptor

Introduction

The first benzodiazepine (BDZ) was synthesised in 1955 by Leo Sternbach [1] when he was working in Basel at Hoffmann–La Roche on the design of new tranquilisers. The project was initially abandoned because the pharmacological properties of the first compounds were disappointing. Two years later, one of the initially synthesised compounds was tested on animal behaviour. Subsequently, the compound showed very strong sedative, anticonvulsant and muscle relaxant effects in humans. These unexpected but impressive findings led, in 1960, to the introduction of chlordiazepoxide into the clinical market under the brand name Librium\textsuperscript{®}. A second compound, diazepam (Valium\textsuperscript{®}), was subsequently marketed in 1963. For a while, these two molecules were the most successful sedative drugs on the market. The introduction of BDZs led to a significant decrease in the prescription of barbiturates [2], which are more toxic and have a higher risk of overdosing and an increased dependence liability. By the 1970s, BDZs had largely replaced the barbiturates previously used for sedation. Since then, nearly thirty different BDZs have been approved and marketed for clinical use.

BDZs enhance the effect of the neurotransmitter \(\gamma\)-aminobutyric acid (GABA), which results in five principal effects that have therapeutic benefits: sedation/hypnosis (for the treatment of insomnia), anxiolysis, control of epileptic seizures, myorelaxation and anterograde amnesia (as a premedication for invasive or otherwise unpleasant medical procedures) [3]. BDZs are classified as either short-, intermediate- or long-acting. Although only subtle pharmacodynamic differences exist, short acting BDZs are preferred for the treatment of insomnia, while the longer-acting BDZs are recommended for the treatment of anxiety. In general, BDZs are safe and effective in the short term. Long-term use remains controversial because BDZs are prone to cause dependence and addiction [4], sometimes referred to as the sixth and unwanted effect [3].

BDZs are allosteric modulators of the GABA\textsubscript{A} receptors, which means that they only act in the presence of the endogenous ligand GABA. Based on efficacy, three groups of BDZs can be distinguished: the positive and the negative allosteric modulators, and the antagonists. The positive modulators potentiate GABA\textsubscript{A}R-evoked currents whereas the negative modulators decrease these currents. The antagonists prevent and reverse the effect of both types of allosteric modulators and have no consequences on the GABA\textsubscript{A}R-mediated currents on their own [3]. Only positive allosteric modulators such as clordiazepoxide (Librium\textsuperscript{®}) and diazepam (Valium\textsuperscript{®}), and the antagonist flumazenil (Anexate\textsuperscript{®}) are used therapeutically. All BDZs bind to a specific cleft located between the \(\alpha\) and the \(\gamma\) subunit of the GABA\textsubscript{A} receptor [5]. Indeed, these ionotropic receptors are \(\mathrm{C}1\) channels composed of five subunits with a specific stoichiometry of 2\(\alpha\), 2\(\beta\) and 1\(\gamma\). Each subunit family exhibits several isoforms: \(\alpha1\)–6, \(\beta1\)–3, \(\gamma1\)–3, and therefore the brain expresses a multitude of GABA\textsubscript{A} receptor combinations. Only \(\alpha1\)-, \(\alpha2\)-, \(\alpha3\)- and \(\alpha5\)-containing GABA\textsubscript{A} receptors are sensitive to BDZs, because they have a crucial histidine residue at homologous positions (\(\alpha1\)H101, \(\alpha2\)H101, \(\alpha3\)H126 and \(\alpha5\)H105) [6]. \(\alpha4\) and \(\alpha6\)
Addiction to benzodiazepines

BDZs were initially greeted with optimism by the medical profession, but, gradually, concerns arose. One major issue is the development of dependence and addiction symptoms after prolonged BDZ treatment [4, 17]. Dependence will occur in anybody who takes an addictive drug and is characterised by specific symptoms that are tolerance and withdrawal. Tolerance manifests when the patient must increase drug dosage in order to feel the same effects. Withdrawal symptoms are observed following discontinuation or abrupt reduction of BDZs dosage, even after a relatively short treatment period (three to four weeks). Such physiological symptoms are the main signs of physical dependence. The most frequent are insomnia, gastric problems, tremors, agitation, fearfulness and muscle spasms [18]. Less frequently observed are irritability, sweating, depersonalisation, hypersensitivity to stimuli, depression, suicidal behaviour, psychosis, seizures and delirium tremens [19, 20]. Over-rapid withdrawal from BDZs also increases the severity of the symptoms. Slow and gradual reduction of dosage customised to the individual accompanied by psychological support are the most effective way of managing withdrawal [21]. Complete withdrawal can require four weeks to several years.

If dependence can be diagnosed in most patients undergoing therapeutic BDZ treatment, the switch to an addicted state only happens in a fraction of drug users (20% for cocaine, less than 10% for BDZs [22]). Addiction is considered a brain disease defined by the World Health Organisation as compulsive substance intake despite negative consequences. It is also characterised by relapse after a prolonged period of abstinence, and so stands apart from dependence. Most often, people who recreationally abuse BDZs also abuse other drugs such as alcohol or opioids (www.nida.nih.gov). In that case, symptoms of dependence and addiction to BDZs manifest faster. For example, patients who have previously abused opioids abuse BDZs more frequently, at larger doses and for longer periods of time compared to BDZ only abusers [23]. However, little work has been done towards understanding cross modulation of pharmacological properties among drugs.

Genetic basis of addiction

As mentioned before not every drug consumer will suffer from addiction, and one hypothesis is that genetic pre-disposition underlies inter-individual vulnerabilities. Studies looking at identical and non-identical twin pairs revealed that when one twin was addicted to alcohol, the other identical twin had a high probability of getting addicted too. However, when a non-identical twin was addicted, the other twin did not necessarily develop an addiction. It was then proposed that addiction is partly due to

**Figure 1**

Mechanism for non-addictive benzodiazepines. (a) The VTA is composed of three major cell types: GABA interneurons (green) and DA neurons (red). Glutamate neurons are not pictured for clarity purpose. Whereas interneurons express α1-containing GABA<sub>A</sub> receptors, DA neurons express GABA<sub>A</sub> receptors that contain other subunits, such as the α3 isoform. In the absence of BDZs, GABA neurons maintain a basal level of inhibition on DA neurons. (b) Clinically used BDZs such as diazepam (Valium<sup>®</sup>) bind to GABA<sub>A</sub> receptors and potentiate inhibitory GABA<sub>A</sub> -evoked-currents on both cell types. However, the overall impact is much stronger in the interneurons because their GABA<sub>A</sub> receptors cause larger unitary currents than the one in DA neurons. Therefore interneurons are hyperpolarised and their activity is decreased. As a consequence, DA neurons are disinhibited and more DA is released, triggering early changes commonly induced by all addictive drugs. (c) α₁-sparing compounds bind to α₁-containing GABA<sub>A</sub> receptors but antagonise the BDZ-binding site (misoriented symbol) and so do not affect the activity of GABA<sub>A</sub> receptors. Hence DA neurons are not disinhibited, preventing an increase of DA release and consequent synaptic adaptations that may eventually lead to addiction.
to genetic factors [24–26]. Another study investigated the first-degree relatives (parents, siblings, or children) of 231 addicts and 61 non addicts. This analysis showed that if a parent had a drug or alcohol addiction, the child had an 8 times greater chance of developing an addiction [27]. Although genetic factors seem to be implicated, environmental influences should not be underestimated. So far there is no clear answer as to which are the sensible genes responsible for addiction, but specific gene mutations have been linked to sensitivity to alcohol and benzodiazepine consumption [28–30].

Another interesting question concerns the change in gene expression after drug abuse. Chronic psychostimulant use is well known to alter neuronal gene expression [31, 32]. Specifically, gene expression after a short cocaine treatment is dependent on CREB (cAMP response element-binding), a ubiquitously expressed protein, whereas gene expression after a long-term cocaine treatment is ΔFosB-dependent [33]. Both CREB and ΔFosB bind to specific DNA sequences, increasing or decreasing the transcription of downstream genes. ΔFosB accumulates in the nucleus accumbens (NAcc) and the striatum after repeated administration of drugs (cocaine, morphine). Several studies support the view that ΔFosB functions as a type of sustained “molecular switch” that gradually converts acute drug responses into relatively stable adaptations that contribute to the long-term neural and behavioural plasticity that underlies addiction [34, 35].

Recently it has been shown that psychostimulants also alter gene expression levels through epigenetic modifications, which means changes affecting the compaction of chromatin and thus DNA sequences’ accessibility to transcription factors. Two of the most studied epigenetic mechanisms are DNA methylation, that tightens chromatin, and histone acetylation, that loosens DNA packaging. In some cases these modifications of the transcriptome are heritable [36].

Cellular basis of addiction

Dependence and addiction symptoms are only observed after repeated drug exposure. However, a single injection is already sufficient to trigger synaptic adaptations in the brain that persist beyond elimination of the compound from the body [37, 38]. This so-called “drug-evoked synaptic plasticity” represents a permissive brain state that may, in the long term, lead towards the development of addiction in vulnerable subjects. Here we review where and how addictive drugs induce such lasting traces even after the first drug exposure.

The ventral tegmental area (VTA), at the origin of the mesolimbic system, consists of three neuronal subtypes: dopamine (DA) neurons, representing the majority of cells in the VTA (70%), γ-Aminobutyric acid (GABA) interneurons (15%, fig. 1a), and recently revealed glutamate neurons (15%) [39]. DA neurons release DA within the VTA and send projections to many different brain regions such as the NAcc, the striatum, the prefrontal cortex and hippocampus. These neurons are excited by unexpected natural rewards and inhibited by both absence of predicted rewards [40] and aversive stimuli [41]. Hence, the VTA is often referred to as the core nucleus of the reward system. GABA interneurons form synapses onto DA neurons and can control their activity by acting as a brake, and also project to the nucleus accumbens [42]. Glutamate cells send axons to the nucleus accumbens and the prefrontal cortex [39], but their role still remains elusive. Addictive drugs have been shown to hijack the mesolimbic reward system by disrupting normal functionality of this neuronal network.

A common particularity of addictive drugs is that they all target the VTA and acutely increase extracellular levels of DA within the VTA and target regions [43, 44]. This step happens within minutes after drug intake and is believed to be at the source of all forthcoming induction of synaptic plasticity and changes in network activity (see below) that happen either few hours after a single injection or after days of chronic drug use [45–47]. Drugs of abuse may therefore be classified by the three cellular mechanisms of action that have been described to cause this acute dopamine increase [48]. Group I includes opioids [49], cannabinoids [50] and γ-hydroxybutyrate (GHB) [51], which decrease the release of GABA from VTA interneurons and thereby remove the inhibitory transmission “brake” onto DA neurons. This indirect increase of DA cells’ activity is known as disinhibition, and is possible due to either cell-type specific expression of their respective receptor to the drug (as in opioids, cannabinoids), or higher affinity of the drug for the receptor located on GABA neurons (GHB). Nicotine constitutes Group II and directly activates DA neurons [52], whereas Group III drugs (including psychostimulants like cocaine and amphetamines) target and perturb the DA transporter (DAT) either by blocking it (cocaine) or reversing its activity (amphetamines) [53].

Neural basis for addictive properties of benzodiazepines

The pharmacological effects of BDZs depend crucially on the α subunit isoform identity. In the VTA, immunohistochemistry has revealed that the α1 subunit isoform is specifically expressed on GABA neurons (fig. 1). In corroboration, unitary currents recorded from GABA neurons are larger and slower than those recorded from DA neurons. The importance of this cellular difference was revealed when midazolam (MDZ, Dormicium®, a non-selective BDZ e.g., modulating all BDZ-sensitive GABA<sub>A</sub> receptors), a BDZ known to act as a positive modulator of GABA<sub>A</sub> receptors, was applied to the slice [3, 16]. The MDZ-induced potentiation of inhibition was stronger onto α1-expressing GABA neurons than onto DA neurons, which silenced the GABA neurons shutting down their transmitter release. As a consequence, BDZ lose their effect on DA neurons, simply because there is no GABA effect anymore to amplify, and the DA neurons are disinhibited (fig. 1b). In vivo, intravenous (iv) administration of MDZ led to a decrease in the firing rate of GABA cells and to an increase in DA neurons activity, thus in DA release. In the α1(H101R) KI mice, MDZ was unable to modulate inhibitory currents in GABA cells. Furthermore no change was observed in either GABA or DA neuron firing rate in vivo [3, 16]. These data indicate that BDZs can be classified as Group I addictive drugs and that they induce an indirect increase in extracellular DA levels by disinhibiting DA neurons (fig. 1b).
To investigate the consequences of the increase in extracellular DA levels, a common experiment is to inject a mouse intraperitoneally (ip) with a drug, slice the brain 24h later and assess synaptic transmission. Initially, it has been shown that after one shot of cocaine, the glutamatergic connection onto VTA DA neurons is strengthened [37]. Furthermore, this strengthening is blocked when the drug is co-administered with a DA D1-like receptor antagonist [46], or when given to a genetically modified mouse carrying a DA transporter insensitive to cocaine, with the result that the drug is unable to induce extracellular DA increase [47]. Since then, these observations have been repeated for all three classes of drugs, showing that this drug-evoked synaptic plasticity is DA-dependent and common to all addictive drugs [47,54]. So far this strengthening of glutamate synapses represents the first known step in the chronology of events triggered by the initial DA increase after a single drug injection and implicated in the development of addiction disease. If there is no further exposure to the drug, the glutamatergic strengthening can go back to baseline after 7 days [37, 54]. However, if the animal is repetitively exposed to the drug, the synaptic plasticity extends to other regions of the brain [55] and other neurotransmitters, as GABAergic transmission onto DA neuron is decreased after 5 days of cocaine [55]. These changes in the mesolimbic network slowly add up and pave the way to addicted behaviour [55,57].

Given the BDZs’ disinhibitory effect on DA neurons and the subsequent strong increase of DA in the target regions as well as in the VTA, it was then investigated if they could evoke any synaptic changes. Again, probing synaptic transmission in the VTA ex vivo and taking advantage of the development of the KI mice, plastic changes was observed by two groups independently [3,16,57]. 24 hours after either ip injection or direct intra-VTA delivery of MDZ, the typical drug-evoked synaptic plasticity was observed onto DA neurons. Coinjection of MDZ with flumazenil, a BDZ antagonist, blocked the plasticity. Interestingly, zolpidem (Stilnox®), an α1 selective non-classical BDZ available in clinics, evoked the plasticity. Conversely, in the α1L(H101R)KI mice the glutamatergic connexion was unchanged after BDZ exposure. Furthermore, ip injection of L838,417 [10], an experimental BDZ, did not induce the plasticity. This compound modulates GABA-evoked currents generated by GABA\textsubscript{A} receptors expressing α2, α3 or α5 subunit isoforms. Importantly it is an antagonist at GABA\textsubscript{A} receptors containing the α1 isoform. This also argues for a specific role of the α1 subunit isoform in mediating addictive BDZ effects.

At a behavioural level, self-administration of addictive drugs, a paradigm that reveals reinforcing properties of a substance, has been shown in several animal models. Oral self-administration of MDZ was tested on WT and α1L(H101R) KI mice. A free choice was given between a drinking bottle containing sucrose and another one containing both sucrose and MDZ. WT mice showed a clear preference for the MDZ-containing solution. However, α1L(H101R) KI animals did not show any preference for the drug-containing solution, indicating that α1-containing GABA\textsubscript{A} receptors are required for the self-administration of MDZ [16]. Altogether, these data indicate that BDZs, like all other addictive drugs, are able to trigger adaptations in the reward system, such as increasing VTA DA cells activity and driving synaptic plasticity of excitatory inputs onto DA neurons. These adaptations depend on the specific expression of α1-containing GABA\textsubscript{A} receptors in GABA neurons of the VTA, mediating early changes induced by BDZs that may eventually lead to addiction.

Conclusions

BDZs continue to be widely used in medical practice for the treatment of anxiety and sleep disorders [59, 60] but are also increasingly popular for recreational purposes. However dependence and addiction are serious side effects, which cannot be neglected. Nevertheless cessation of BDZ prescription would be the waste of a valuable therapeutic tool. The results in animal models that we have reviewed here offer opportunities for the development of novel drugs addressing these side effects. It has been shown that α1-containing GABA\textsubscript{A} receptors mediate the addictive properties of BDZs. The α1-sparing compound L838,417 does not induce the changes in the brain that are observed with clinically used BDZs and other addictive drugs [16] and thus, L838,417 would be predicted to have no or at least a significantly reduced addictive potential compared to clinically used BDZs (fig. 1c). Unfortunately, the development of L838,417 was aborted due to unfavourable pharmacokinetic parameters in rodents [61]. Another fact to take in account is that α1-containing GABA\textsubscript{A} receptors outside the VTA are presumably necessary for classical BDZs to induce sedative and anticonvulsant effects. Thus, α1-sparing compounds would be predicted not to exhibit these effects, but would still be efficient anxiolytics, an effect mediated by α2-containing GABA\textsubscript{A} receptors. α1-sparing compounds might therefore represent non-sedating anxiolytics.

In any case, we believe that effort should be directed towards the development of α1-sparing compounds to render them safe and effective for future clinical use. The preclinical data reviewed here would suggest that such drugs provide many of the therapeutic benefits of BDZs, would likely be devoid of sedative side effects (which is actually useful when used for the treatment of anxiety disorders) and most importantly, lack addictive liability.

Funding / potential competing interests: Funding: National Centers of Competence in Research. The authors have no conflict of interest relevant to this article.

Correspondence: Kelly R. Tan, PhD, Department of Basic Neurosciences, Medical Faculty, University of Geneva, CH-1211 Geneva, Switzerland, kelly.tan@unige.ch

References


Mechanism for non-addictive benzodiazepines.

(a) The VTA is composed of two major cell types: GABA interneurons (green) and DA neurons (red). Glutamate neurons are not pictured for clarity purpose. Whereas interneurons express α1-containing GABA<sub>A</sub> receptors, DA neurons express GABA<sub>A</sub> receptors that contain other subunits, such as the α3 isoform. In the absence of BDZs, GABA neurons maintain a basal level of inhibition onto DA neurons.

(b) Clinically used BDZs such as diazepam (Valium®) bind to GABA<sub>A</sub> receptors and potentiate inhibitory GABA<sub>A</sub> evoked-currents on both cell types. However, the overall impact is much stronger in the interneurons because their GABA<sub>A</sub> receptors cause larger unitary currents than the one in DA neurons. Therefore interneurons are hyperpolarised and their activity is decreased. As a consequence, DA neurons are disinhibited and more DA is released, triggering early changes commonly induced by all addictive drugs.

(c) α1-sparing compounds bind to α1-containing GABAA receptors but antagonise the BDZ-binding site (misoriented symbol) and so do not affect the activity of GABA neurons. Hence DA neurons are not disinhibited, preventing an increase of DA release and consequent synaptic adaptations that may eventually lead to addiction.